Unc l

SECURITY CLAS

AD-A200 374

MENTATION PAGE

1a. REPORT SE: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; [illegible]mited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) University of Colorado Boulder, CO 80309	5. MONITORING ORGANIZATION REPORT NUMBER(S) AFOSR-TR- 88-1092
6a. NAME OF PERFORMING ORGANIZATION University of Colorado Boulder, CO 80309 (6b. OFFICE SYMBOL (If applicable))	7a. NAME OF MONITORING ORGANIZATION Air Force Office of Scientific Research
6c. ADDRESS (City, State and ZIP Code) Institute for Behavioral Genetics Box 447 Boulder, CO 80309	7b. ADDRESS (City, State and ZIP Code) Building 410 Bolling Air Force Base, DC 20332-6448
8a. NAME OF FUNDING/SPONSORING ORGANIZATION AFOSR (8b. OFFICE SYMBOL (If applicable) NL)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER AFOSR-85-0369
8c. ADDRESS (City, State and ZIP Code) Building 410 Bolling AFB, DC 20332	10. SOURCE OF FUNDING NOS. PROGRAM ELEMENT NO. 61102F; PROJECT NO. 2312; TASK NO. A2; WORK UNIT NO.
11. TITLE (Include Security Classification) Behavioral Consequences of Neurotransmitter Regulation	
12. PERSONAL AUTHOR(S) Jeanne M. Wehner	
13a. TYPE OF REPORT Final	13b. TIME COVERED FROM 9-15-85 TO 9-14-88
14. DATE OF REPORT (Yr., Mo., Day) 9-1-88	15. PAGE COUNT 6
16. SUPPLEMENTARY NOTATION	
17. COSATI CODES: FIELD / GROUP / SUB. GR.	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of the research was to determine whether the status of brain muscarinic cholinergic receptors have a role in the mediation of behavioral responses. The behavior of choice was spatial reference memory as defined using the Morris water task. Subjects for the testing were different inbred strains of mice. An initial screen showed variability in learning ability using various strains. The major portion of the work used C57BL mice which showed good learning ability and DBA/2J mice which were poor learners. Chronic treatment with DFP produced only a learning deficit in the C57BL mice and only when administered before acquisition began. Likewise, chronic treatment with oxotremorine had a similar effect indicating that a functional cortical/hippocampal cholinergic system is important during early stages of learning.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS ☐	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr William O. Berry	22b. TELEPHONE NUMBER (Include Area Code) (202) 767-5021 / 22c. OFFICE SYMBOL NL

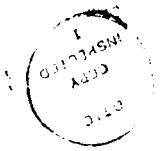
DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE. Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

26 AUG 1988

19. (Cont)

The cholinergic cortical and hippocampal systems of C57Bl and DBA/2J mice were examined. They do not differ in the localization of muscarinic receptors, but do differ in acetylcholinesterase activity. Furthermore, they differ in hippocampal protein kinase C, a proposed mediator of long-term potentiation and learning. We believe the protein kinase C difference in important because a series of C57BL X DBA/2 recombinant inbred strains differ in learning ability and protein kinase C activity. We have observed a positive and significant correlation between these two parameters. Our current hypothesis is that a deficit of hippocampal protein kinase C produces a learning deficit in DBA/2 mice.



FINAL REPORT

Behavioral Consequences of Neurotransmitter Receptor Regulation:
Principal Investigator: Jeanne M. Wehner

1) Summary :

Spatial learning ability using the Morris water task was assessed in inbred strains of mice. After initial characterization, two strains were selected to perform studies, C57BL mice which performed the task well, and DBA2/J mice which were impaired in their performance. A comparison of cholinergic markers indicated a significant difference between these two strains in acetylcholinesterase activity and in hippocampal protein kinase activity. The difference in protein kinase activity appears to relate to their differing learning ability because a significant correlation between learning ability and hippocampal protein kinase C activity was observed in recombinant inbred strains generated from a cross of C57BL and DBA/2J mice. Additional pharmacological studies were performed in which cholinergic receptors were manipulated by either chronic treatment with an anticholinesterase or an agonist. Such treatments produced a decrease in muscarinic receptors and an impairment in acquisition of spatial learning. These studies demonstrate that cholinergic systems are important during initial acquisition of spatial learning and that coupling of receptors via activation of protein kinase C activity may be an important determinant of learning ability.

2) Statement of Work:

The following summary is provided to supplement the enclosed reprints. Our objectives were to determine strains of mice that showed the ability to perform a spatial learning task and then to use spatial learning as a behavioral test to understand the role of cholinergic receptor regulation in spatial learning by manipulating receptor numbers in brain regions of interest i.e., the cortex and hippocampus. Also of interest was the genetic regulation of spatial learning behaviors.

1) Strains survey of spatial learning: In order to pick strains of mice that would be useful in exploring genetic variation in the regulation of learning and memory, we conducted a strain survey. Strains were screened using the Morris water task on cue learning and place learning. Cue learning was used as an indicator of visual acuity, because some inbred strains have visual deficits that might prevent them from undergoing spatial learning. As we had anticipated, some strains could not perform this task because of visual problems caused either by the expression of a retinal degeneration gene or by albinism. A summary of the inbred strain survey is presented below:

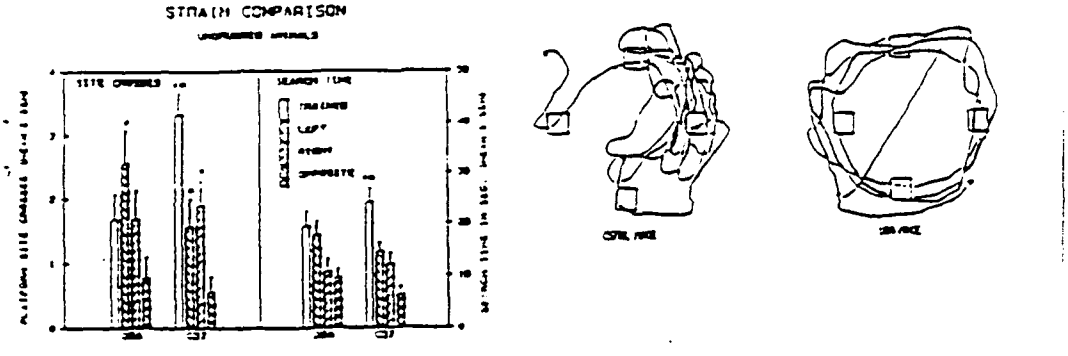
Table 1

Good Learners: C57BL/6 Ibg, C57BL/10J, LS females, C57BL/J
Poor Learners: DBA/2 Ibg or /2J, LS males, DBA/1J, LP/J
Blind: SWR/J, SJL/J. RIII/J, P/J, CBA/J. C3H/Ibg, BALB/cByJ

We more thoroughly characterized the BALB/c, C57BL/6, DBA/2, and C3H

strains. The results of these studies were published in Behavioral Genetics 18: 55-68, 1988. Of these four strains, C57BL/6 and DBA/2 mice can learn to locate the visible platform and become better in locating the visible platform as a function of trials, while the albino BALB/c mice and C3H mice carrying the retinal degeneration gene do not. C57BL/6 and DBA/2 mice could be distinguished in their ability to perform place learning as reflected in both latency to reach criterion and preference as measured by a probe-trial in which the platform in removed and the pattern of the animals swimming is videotaped after the final trial. The tapes are then scored for site crossing or the number of times as animal crosses the site to which it was trained or the three other possible sites. Another measure is also taken to determine the time spent searching the quadrant that held the trained site and all other quadrants (search time). These scores are shown in Figure 1; C57BL mice showed a preference for the trained platform both measures in the probe trial while DBA/2 mice did not show this preference. A typical path tracing during the probe trial for each strain is also shown. C57BL/6 mice concentrate their effort near the site to which they were trained while DBA/2 mice use a search method known as a taxon strategy whereby they do cross the platform site but show no preference for the trained site.

FIGURE 1



2) Cholinergic studies in C57BL and DBA/2 mice: Since a role of cholinergic systems has been implicated in spatial learning, we used an anticholinesterase, diisopropylfluorophosphate (DFP), to modifiy cholinergic function. We treated C57BL and DBA/2 mice with DFP (2mg/kg) every other day for 12 days, either before acquisiton training or after training. When C57BL mice were treated with DFP prior to acquisition training they were impaired in their ability to undergo spatial learning as measured by both latency scores and lack of preference for the trained site (Figure 2A). They were not impaired on the visible platform task. Figure 2B shows the path tracing of control and DFP-treated C57BL mice indicating this impairment. The results of the C57BL study were reported in Pharmacol. Biochem. Behav. 27: 143-151, 1987.

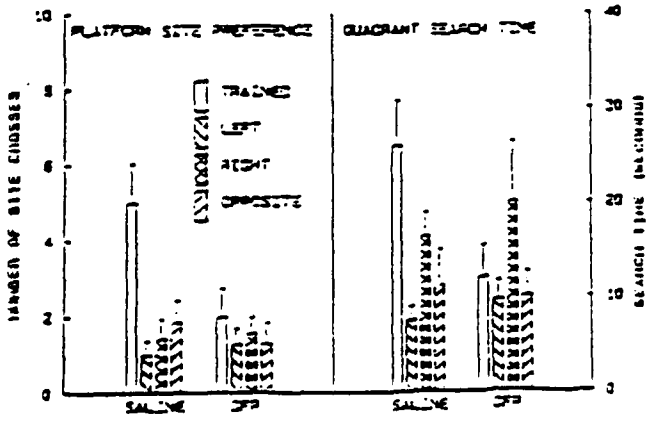
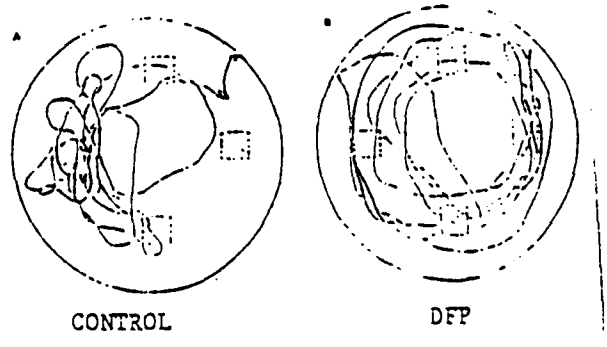


FIGURE 2

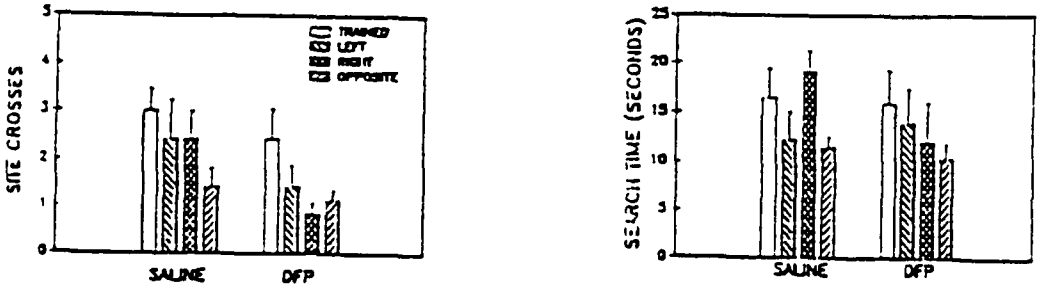


This effect of DFP was fairly long-lasting, such that when C57BL mice were tested 16 days later on retention, they still were impaired and could not perform a reversal task in which they were required to find a new platform position. When DFP was administered after training, it was without effect, indicating that DFP does not impair retention. This suggests that functional integrity of the cholinergic system is important for acquisition of new information.

The effects of DFP on muscarinic receptor number in C57BL/6 mice were examined and as expected, DFP produced a loss of muscarinic receptors in cortex, hippocampus, striatum, and hypothalamus during the time of initial training the time of retention training (16 days), receptors had returned to normal (see enclosed reprint). Thus, the inital impairment in spatial learning ability was associated with a loss of cortical and hippocampal muscarinic receptors, regions believed to be important in spatial learning during the acquisition period. Futhermore, the inability of mice to use spatial cues during the reversal task at a time period when receptors had returned to normal suggested that once an animal has learned the task using a nonspatial strategy, it cannot switch to a form of spatial strategy at a later time.

In contrast to the results of DFP treatment of C57BL mice, DBA/2 mice showed a different pattern after chronic DFP treatment. These results are described in the enclosed reprint from Pharm. Biochem. Behav. 29: 325-329, 1988. Specifically, DBA /2 mice were not impaired by DFP treatment as shown in probe-trial scores (Figure 3). In fact, there appears to be a trend toward improvement in the DFP-treated mice, a subject which we are pursuing at this time because this may provide additional evidence for a cholinergic malfunction in the DBA/2 mice. DFP produced a loss of brain muscarinic receptors in DBA/2 mice and it may be that decreasing the number of receptors actually leads to improvement by adjusting a cholinergic imbalance in DBA mice. The results of the DFP experiments suggest that DBA/2 mice do not use a cholinergically based strategy to learn the Morris water task.

FIGURE 3



To substantiate further that the cholinergic system is important for acquisition of spatial learning, we examined the effects of chronic treatment

with a direct muscarinic agonist, oxotremorine (OXO). OXO also produces down-regulation of muscarinic receptors but via a direct mechanism, i.e., binding at the receptors. The results of this study are described in the enclosed manuscript that is in press in Pharm. Biochem. Behav.. Mice were treated with OXO by continuous subcutaneous infusion, removed from treatment and began training in the Morris water task. When the animals began acquisition training 24 hrs after their removal from oxotremorine t atment, oxotremorine-treated animals performed poorer than the saline-treated controls in the probe trial test which is conducted on the fourth day after treatment. In contrast, animals that were trained beginning at 48 hrs after cessation of treatment, were no longer impaired and performed like saline-treated mice in the probe-trial test. Unlike the DFP treatment, OXO produced a less severe impairment in that it was short-lived. It appears from these data that deficits were present only when receptors were reduced during both acquisition. We conclude from these studies that the status of the cholinergic system is important during initial acquisition and that there must be some finite period of time during which reduced receptor function is important in regulation of spatial learning.

We and our collaborators have performed comparisons of C57BL and DBA/2 mice on several cholinergic markers including: choline acetyltransferase (ChAT) activity, acetylcholinesterase (AChE), and the number of muscarinic or nicotinic receptors. There is no difference in ChAT activity in crude cortical homogenates between C57BL and DBA/2 mice.

As observed by others, DBA/2 mice have more AChE activity in cortex and hippocampus than do C57BL mice (Table 2). This may indicate that less acetylcholine is present in the synapse, but we will not know whether this difference is important to their differing spatial learning abilities until we perform a correlational analysis using the recombinant inbred strains.

Table 2
(umole/hr/mg protein)

	Cortex	Hippocampus
C57BL/6Ibg	4.37 ± 0.25	2.81 ± 0.25
DBA/2Ibg	7.26 ± 0.30	4.16 ± 0.17

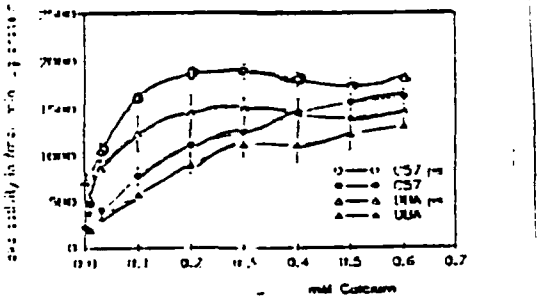
We have examined high affinity choline uptake in cortex and hippocampus of C57BL and DBA/2 mice and have observed no significant differences. There are more muscarinic receptors in cortical membrane preparations of DBA/2 mice (3780.8 ± 86.9 fmole/ mg protein) as compared to C57BL mice (3119.8 ± 223.9 fmole/ mg protein) as measured by QNB binding ($p<0.05$). When we analzyed this difference more thoroughly by receptor localization studies using quantitative autoradiography, we observed no significant differences in cortical or hippocampal areas.

3) Coupling phenomena for the cholinergic systems: Because the differences between C57BL and DBA/2 mice at the level of the muscarinic receptor are rather unspectacular, we have recently begun to examine receptor coupling mechanisms. We have recently established the phosphatidyl inositol turnover and protein kinase C methods of analysis in our laboratory. Activation of some muscarinic receptors stimulates membrane phosphatidylinositol (PI) turnover, thereby liberating diacylglycerol and IP_3. This process leads to an

activation of protein kinase C (PKC) which catalyzes the phosphorylation of intracelllar substrates. Hippocampal PKC has been implicated in long-term potentiation and therefore may be an important determinant of learning ability.

While no data is available on PI turnover as yet, the PKC experiments have provided interesting results. We have examined brain PKC in C57BL and DBA/2 mice by dose-response analysis of Ca^{++} and phosphatidyl serine (PS) activation of cortical and hippocampal PKC in cytosolic and membrane fractions. As shown in Figure 4, C57BL have higher basal and activated PKC in hippocampal membranes than DBA/2 mice ($p<.001$). This appears to be specific to hippocampal membranes because the two strains do not differ in cortical cytosolic or membrane PKC. This is a very exciting observation, and to our knowledge is the first demonstration of a genetic difference in hippocampal PKC. Because brain PKCs are encoded by a family of genes and in some cases the multiple types of brain PKC activity are distinguished by their differing sensitivty to Ca^{++} or phosphatidyl serine activation, it maybe that the difference between C57BL and DBA/2 mice represents differences in the PKC genes, expression of these genes, or presence of an endogenous inhibitor of PKC activity in DBA/2 brain tissue. Funding to continue this aspect of the work is now being sought from other sources.

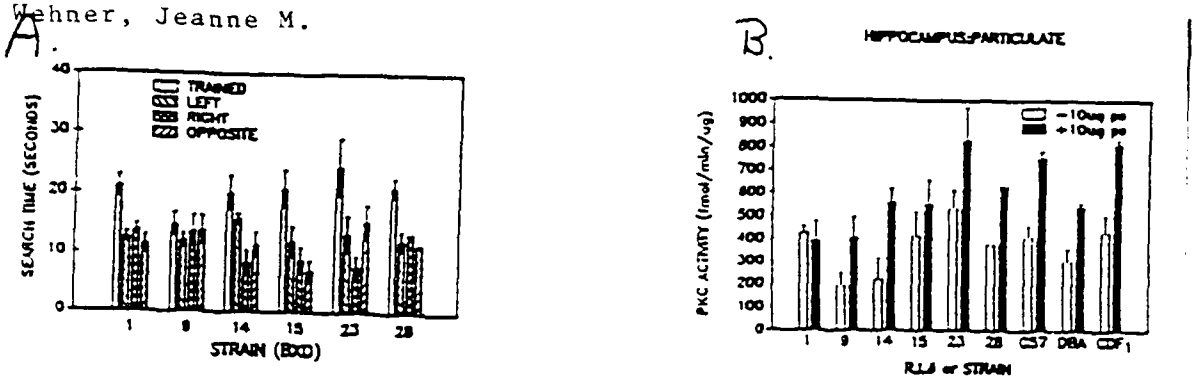
FIGURE 4



We are now attempting to examine whether this difference correlates to differing learning and memory capabilities and have begun screening 27 recombinant inbred strains (RIs) on spatial learning ability and PKC activity. After screening only 6 RIs, we have observed that there is variation in learning ability as well as PKC activity. Figure 5A shows the probe-trial data from these RIs after training in the Morris water task. At least one of these strains [#9] shows a lack of preference for the trained site. PKC activity of hippocampal membranes (Figure 5B) correlates with the accuracy of learning as indicated by a positive correlation of 0.82 ($p < .05$) between particulate Ca^{++}, PS-stimulated PKC activity and a site preference score. Again this variation is specific because cortical PKC does not differ among the strains. These data demonstrate that there may be a correlation with PKC activity in hippocampal membrane and learning ability. We are currently expanding this analysis to additional RIs and examining whether this correlation is specific to site-crossings. We are also addressing what neurotransmitter system may serve to activate this particular hippocampal PKC.

FIGURE 5

(next page)



4) Genetic analysis of spatial learning in crosses of C57 and DBA mice: Having two inbred strains of mice that differ in spatial learning ability allows one to examine the genetic regulation of this behavior. We have completed a classical genetic analysis of spatial learning using crosses of the good learners, C57BL mice, with the poor learners, DBA mice (see enclosed submitted manuscript). We have analysed inheritance patterns for three measures: total latency, site-crossings and quadrant search time. A genetic analysis was performed for these three measures to assess dominance, additivity, and epistasis. All three measures of spatial learning showed different inheritance patterns and therefore should be useful as the biochemical correlational analysis proceeds. The genetic analysis of total latency scores across all trials required a complex model that included interactions of sex with additivity, dominance deviation and epistasis. In fact, the fit of the model for this measure was forced by the constraints of the model and is probably too complex of a measure because multiple genes may regulate this aspect of the behavior. We were more successful in fitting the other two measures. There were sex differences, with females having a more complicated pattern. Only the data from the males are shown here and only males will be used in the future. To analyze search time and site crossings, we derived one score, a preference score which is defined as the number of correct crosses or time spent in the trained quandrant minus the mean of the incorrect crosses at other sites or time spent in untrained quadrants. In figure 6 is shown the site crossing and search time preference scores for male mice. All the derived crosses were better than DBA/2 mice and the F_1 hybrids were better than either parental strain. This phenomenon is called heterosis or hybrid vigor. The parameter estimates were not identical for the two measures, but by assessing the dominance to additivity ratio for both search time and site crossing, it was observed that the genetic pattern was very similar and that the inheritance pattern could be explained entirely by the action of dominant genes. Because of these simpler patterns for site crossing and search time in males, we are hopeful that we will be able to correlate a biochemical parameter with one or both of these measures. The RIs will allow an independent confirmation of these observations and if there are two different genes regulating search time and site crossing, we should be able to observe this in the RIs.

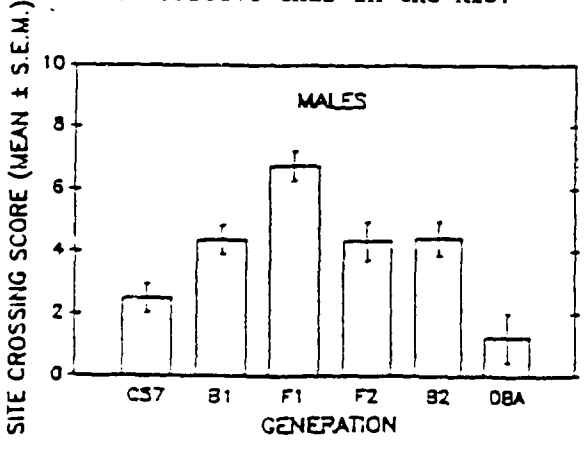
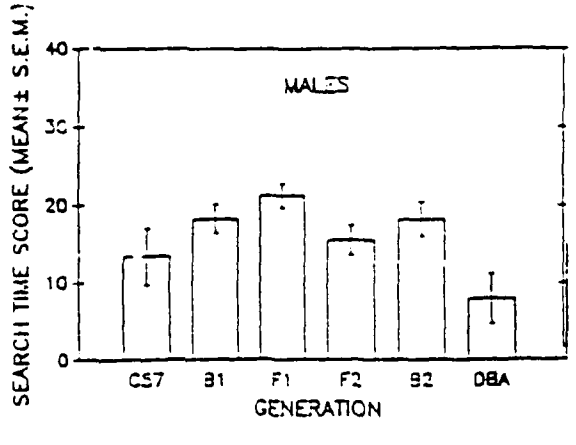


FIGURE 6



5) Effects of Glutamate anatgonists on spatial learning: Morris and Lynch observed that animals treated with the glutamate antagonist, AP5, have impaired spatial learning ability. A new antagonist, CPP, that can be adiminstered peripherally has become available and might be a useful tool to examine further strain differences in spatial learning and the systems mediating these differences. We administered CPP each day before training and determined the affects on spatial learning ability. The results indicate that a clean easily interpreted effect of CPP is not observed. In fact small changes in the protocol results in normal performance after CPP treatment and clearly indicate that not all glutamate antagonists produce the impairments observed with AP5. A more detailed description of these data is found in the enclosed manuscript.

3)Status of the Research: We have made several important observations during the course of this funded research including: 1) Mouse strains differ in spatial learning ability and some strains carry genes that prevent them from showing a perference for a trained site. These animals provide an important model to investigate mechanisms underlying the neurochemical regulation of spatial learning. 2) The status of the cholinergic system during acquisition of learning is important. 3) The inability to learn as demonstrated by the DBA/2 mice may be related to a lower activity of protein kinase C in the hippocampus. This observation supports recent hypotheses that PKC activation may be required for longer term maintenance of learning. 4) Different aspects of spatial learning are regulated by different genetic systems. By using appropriate genetic populations of mice, the genes regulating these behaviors may be characterized.

4) List of publications:

1.) Marks MJ, Stitzel JA, Romm E, Wehner JM, Collins AC: Nicotinic binding sites in rat and mouse brain: Comparison of acetylcholine, nicotine, and alpha-bungarotoxin. Molecular Pharmacology 30: 427-436, 1986.

2.) Upchurch M, Wehner JM: Effects of chronic diisopropylfluorophosphate treat-ment on spatial learning in mice. Pharmacology Biochemistry and Behavior 27: 143-151, 1987.

3). Upchurch M, Wehner JM: Differences between inbred strains of mice in Morris water maze performance. Behavior Genetics, 18: 55-68, 1988.

4.) Upchurch M, Wehner JM: DBA/2Ibg mice are incapable of cholinergically-based learning in the Morris water task. Pharmacology Biochemistry and Behavior 29: 325-330, 1988.

5.) Wehner JM, Upchurch M: The effects of chronic oxotremorine treatment on spatial learning and tolerance development in mice. Pharmacology Biochemistry and Behavior, in press.

6.) Collins AC, Romm E, Wehner JM: Nicotine tolerance: An analysis of the time course of its development and loss in the rat. Psychopharmacology, in press.

7.) Upchurch M, Wehner JM: Heterosis and resistance to DFP effects on spatial learning in C57BL X DBA hybrids, Brain Res. Bull., in press.

8.) Upchurch M. and Wehner, JM: Inheritance of spatial learning ability in inbred mice: A classical genetic analysis. Behav. Neurosci. submitted.

9.) Upchurch M, Wehner JM: CPP effects on spatial learning ability in mice: Interaction of training protocol and N-methyl-D-aspartate receptor antagonism. Psychopharmacol., submitted.

In preparation:

10.) Wehner JM, and Sleight S: Strain differences in hippocampal protein kinase C activity. to be submitted to Brain Research.

11.) Upchurch M. and Wehner JM: A genetic analysis of spatial learning ability in recombinant inbred strains of C57BL and DBA/2J mice. to be submitted to Brain Research.

5)Professional Personnel

Jeanne M. Wehner, Associate Professor, Institute for Behavioral Genetics, University of Colorado.

Allan C. Collins, Professor, School of Pharmacy and Institute for Behavioral Genetics, Unversity of Colorado.

Margaret Upchurch, Research Associate, Institute for Behavioral Genetics, University of Colorado.

6)Interactions:

"Genetic and mechanistic studies of spatial learning" by Margaret Upchurch, Behavior Genetics Society, Minneapolis, Minnesota, June 1987.

"Heterosis and resistance to DFP in F1 hybrids of C57BL and DBA/2J mice" by Jeanne M. Wehner, International Behavior Genetics meeting, Catholic University, Netherlands, June 1988.